What is claimed is:

- 1. A method of preparing a pro-urokinase ("pro-UK") mutant polypeptide, the method comprising
 - (a) obtaining a nucleic acid molecule that encodes a pro-UK mutant polypeptide;
- (b) inserting the nucleic acid molecule into a pET29a expression plasmid comprising a phage T7 promoter and Shine-Dalgarno sequence;
- (c) transforming *E. coli* type B strain bacteria BL21/DE3 RIL with the expression plasmid;
- (d) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express pro-UK mutant polypeptide; and
 - (e) isolating the pro-UK mutant polypeptide from the transformed bacteria.
- 2. The method of claim 1, wherein the pro-UK mutant is a pro-UK flexible loop mutant.
- 3. The method of claim 2, wherein the pro-UK flexible loop mutant comprises the mutation Lys³⁰⁰ \rightarrow His.
- 4. The method of claim 1, wherein the pro-UK mutant is non-glycosylated and has a molecular weight of about 45,000 daltons.
 - 5. The method of claim 1, wherein culturing comprises a two-stage fermentation.
- 6. The method of claim 5, wherein the first stage of fermentation comprises adding to a flask a cell culture diluted in sterile EC1 medium and growing the culture at about 34 to 37°C for at least about 10 hours with agitation to form a seed culture, wherein the cell culture comprises a glycerol suspension of an LB culture of the transformed bacteria and containing a sufficient amount of kanamycin.
 - 7. The method of claim 5, wherein the second stage of fermentation comprises
 - a) adding the seed culture to a fermentor;
 - b) maintaining the pH in the fermentor at about 6.8 to 7.2;

- c) maintaining the dissolved oxygen concentration in the culture medium at about 35 to 45% of air saturation;
 - d) maintaining the temperature of fermentation at about 34 to 37°C; and
- e) adding to the fermentor a nutrient feeding solution comprising one or more sugars when all glucose initially present in the fermentor at step a) is consumed, following the equation $V = Vo e^{0.18t}$, where V = volume of feeding solution added (ml/h), Vo = 1/100 of the starting fermentation medium (ml), and t = time of fermentation after the start of the feeding phase (hours).
- 8. The method of claim 1, wherein the expression plasmid containing the nucleic acid molecule is pET29aUKM5.
- 9. The method of claim 1, further comprising preparing two-chain pro-UK mutant by passing the pro-UK mutant over plasmin bound to a substrate.
- 10. The method of claim 9, wherein the substrate is an agarose-based gel filtration medium.
- 11. The method of claim 1, further comprising combining the isolated pro-UK mutant polypeptide with an acidic excipient.
- 12. A composition comprising an isolated, single-chain pro-urokinase ("pro-UK") mutant polypeptide produced according to the method of claim 1, wherein at least 96% of the protein in the composition is the single-chain pro-UK mutant polypeptide.
- 13. A composition of claim 12, wherein at least 98% of the protein in the composition is the pro-UK mutant polypeptide.
- 14. The composition of claim 12, wherein the pro-UK mutant polypeptide is a pro-UK flexible loop mutant.
 - 15. The polypeptide of claim 12, wherein the pro-UK mutant is M5.
- 16. The composition of claim 12, further comprising a pharmaceutically acceptable excipient.

- 17. A composition of claim 16, wherein the pharmaceutically acceptable excipient is an acidic excipient.
- 18. A composition comprising an aliquot of 20 to 40 mg of a pro-UK mutant made by the method of claim 1, packaged with directions for use in administering as a bolus to a patient exhibiting symptoms of a stroke or a heart attack.
- 19. A purified culture of *E. coli* type B strain bacteria BL21/DE3 RIL, wherein bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide.
 - 20. The culture of claim 19, wherein the expression plasmid is pET29aUKM5.
- 21. A method of preparing a pro-urokinase ("pro-UK") mutant polypeptide, the method comprising
- (a) obtaining a transformed bacteria, wherein the bacteria is an E. coli type B strain bacteria BL21/DE3 RIL transformed with a pET29a expression plasmid comprising a phage T7 promoter, a Shine-Dalgarno sequence, and a nucleic acid molecule that encodes a pro-UK mutant polypeptide;
- (b) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express pro-UK mutant polypeptide; and
 - (c) isolating the pro-UK mutant polypeptide from the transformed bacteria.
- 22. The method of claim 21, wherein the pro-UK mutant is a pro-UK flexible loop mutant.
- 23. The method of claim 22, wherein the pro-UK flexible loop mutant comprises the mutation $Lys^{300} \rightarrow His$.
- 24. The method of claim 21, wherein the expression plasmid containing the nucleic acid molecule is pET29aUKM5.